at pH 2.0) reported by Allen, Hogan, and Rothschild from radiation results.[23]

Finally, we should emphasize that our conclusions as to a redox chain process apply only to aquo Fe(III) in acid solution. Complexed Fe(III) in less acid media catalyzes H_2O_2 decomposition with rates which vary enormously with the ligands present, and often leads to concurrent oxidation of organic substrates.[7,24] While these reactions have usually been interpreted in terms of "complex" mechanisms, redox chains remain a possibility,[24] and we hope to extend our techniques to such systems in the future.

Experimental Section

Materials. Stock solutions of reagents were prepared from analytical grade perchlorate and nitrate salts, perchloric and nitric acid, 30% H_2O_2, distilled under reduced pressure, and commercial organic reagents. All reaction mixtures were adjusted to an ionic strength of 0.435.

Kinetic Measurements. Reactions were carried out in magnetically stirred vessels thermostated at 30° or other temperatures, and followed by withdrawing aliquots at intervals, and quenching in dilute H_2SO_4 and titrating H_2O_2, usually with $KMnO_4$. Preliminary experiments without retarders gave decomposition rates in good agreement with literature results. Gas evolution experiments were carried out in similar vessels, previously saturated with O_2, connected to a gas-buret. After equilibration of the other components, H_2O_2 was injected into the system through a rubber septum. Ratios of rate constants cited were all obtained from least-squares fits to linear plots such as those shown, using initial values for peroxide decomposition.[25]

Product analyses were generally carried out on systems at substrate/H_2O_2 ratios of 4. After reaction Fe^{3+} was removed either by treatment with Dowex 50 W-X8 (H^+ form) ion exchange resin, or neutralized with NaOH and filtered. Solutions were analyzed by flame ionization glc and products determined by isolation or retention times in comparison with known standards. Hydroxyacetone was further identified by tlc comparison of its 2,4-dinitrophenylhydrazone with authentic materials; organic peroxides were detected by tlc as described by Cartilidge and Tipper.[26]

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Synthesis of Tritium- and Deuterium-Labeled Apomorphine[1a]

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Abstract: Tritium-labeled apomorphine was synthesized from morphine and apomorphine with specific activities of 3.62×10^3–1.88×10^5 dpm/μg with labeling occurring predominantly on the aromatic rings as demonstrated by pmr and mass spectroscopy studies of apomorphine deuterated under identical conditions. By taking advantage of the large differences in exchange rates of various aromatic hydrogens as shown by pmr studies, one may label exclusively the catechol ring of apomorphine by controlling the reaction conditions.

The action of apomorphine (I) in alleviating the symptoms of parkinsonism[2] may be due to its structural analogy with 3,4-dihydroxyphenylethylamine (II)[3] and phenylethylamine (III). II acts as a dopaminergic neurotransmitter while phenylethylamine displaces neurotransmitters from cellular sites.[4] Some of the beneficial effects of 3,4-dihydroxyphenylalanine, a precursor of II,[5] are suspected to arise from its participation in the synthesis of tetrahydropapaveroline (IV) or the isomeric tetrahydroxynoraporphines (Va and Vb) which are analogs of I.[6] In turn, the structural relationship between I, IV, Va, and Vb suggests that some of the pharmacological properties of I may be due to its *in vivo* hydroxylation in ring A. In I the rings A and D constitute a biphenyl system which has been the subject of hydroxylation studies *in vitro* and *in vivo*.[7]

To test some of the above ideas and to study the distribution of I among animal organs and intracellular organelles, it became necessary to synthesize labeled

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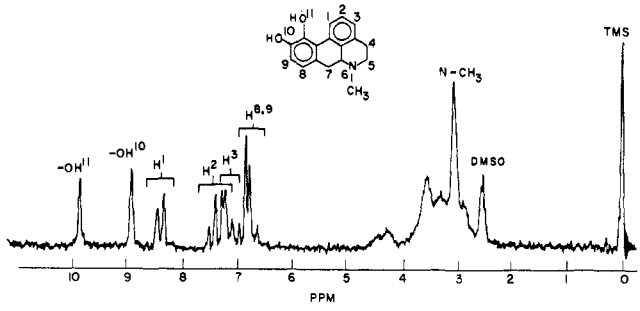
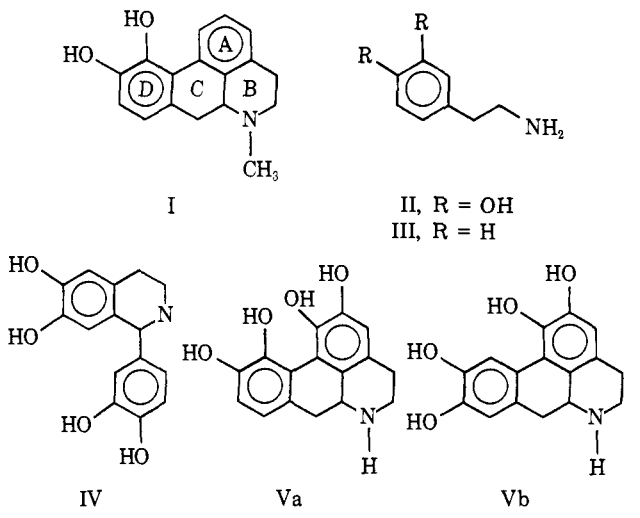


Figure 1. The 60-MHz pmr spectrum of apomorphine in dimethyl-d_6 sulfoxide with tetramethylsilane as an internal standard.



I

II, R = OH
III, R = H

IV Va Vb

apomorphine. In this report we describe the synthesis of tritiated apomorphine hydrochloride (VI) and the spectral analysis of similarly prepared deuterated I to define the labeled sites. We have also explored the possibility of selective labeling through the control of reaction conditions.

Results

By using tritiated H_3PO_4 in the preparation of [^{3}H]-apomorphine hydrochloride (VI) from morphine base,[8] the tritiated compound was obtained in 8–17% yields, purified and lyophilized to constant specific activities ranging between $3.62 \pm 0.03 \times 10^3$ and $1.65 \pm 0.01 \times 10^5$ dpm/μg depending on the specific activity of the TH_2PO_4. The low yields of VI are due to the polymerization and the formation of degradation products of morphine under the reaction conditions employed.[9] VI was also prepared from I by acid catalysis in a homogeneous phase using the above isolation procedure and method of purification. The yield was 21% and the specific activity $1.88 \pm 0.02 \times 10^5$ dpm/μg. All labeled compounds were indistinguishable from I by tlc and spectrophotofluorimetry.

A detailed assignment of the lines in the pmr spectrum (Figure 1) of apomorphine has been made for following the site and rate of deuteration. The NCH_3 resonance was readily recognized as the three-proton singlet at 2.45 ppm;[10] in the spectrum of apomorphine hydrochloride this peak shifted downfield by 0.6 ppm.

Double resonance experiments using the frequency decoupling technique showed that the lines in the 6.90–8.28-ppm region are due to three intercoupled protons, obviously H-1, H-2, and H-3.[11]

The broadened doublet at the extreme low aromatic region, centered at 8.21 ppm, was assigned to H-1 rather than to H-3 since the former alone is subject to the deshielding effect of the phenolic hydroxyl in its immediate neighborhood and the ring-current effect of the D ring.[10,12] Irradiation at the resonance frequency of H-1 simplified the spectrum in the 6.90–7.31-ppm region to a quartet (AB pattern[13] due to H-2 and H-3, J_{23} = 7.7 Hz). Further decoupling experiments indicated that J_{12} = 7.1 Hz and J_{13} = 1.0 Hz. The approximate chemical shifts of H-1, H-2, and H-3 were estimated to be 8.21, 7.17, and 7.00 ppm, respectively. Distinction between H-2 and H-3 was easy because the former is coupled to two ortho protons whereas the latter is subject to one large (ortho) and one small (meta) coupling.[14] With the aid of a computer program, LAOCOON 3,[15] an analysis of the ABX spectrum due to H-1 H-2, and H-3 yielded the following chemical shifts in ppm: 8.20, 7.17, 7.00; with coupling constants in Hz: $J_{12} = 7.40, J_{13} = 0.93, J_{23} = 7.77$.

The remaining four lines in the aromatic region must correspond to the AB pattern due to H-8 and H-9. An analysis of this AB pattern gave 6.73 and 6.60 ppm as the chemical shifts of the two protons. A scan of the quartet over a sweep width of 100 Hz showed clearly that the higher field proton was subject to a small long-range coupling. Inspection of a "Dreiding model" of I showed that the 7β-H which is benzylic to H-8 forms an "allylic angle" with it of about 90°. Under these conditions, J_{78} would be maximal while J_{79} would be nil.[16] The higher field proton (6.60 ppm) must be assigned therefore to H-8.

The hydrochloride of the deuterated I was prepared from I under the same reaction conditions as VI but using 85% D_3PO_4 obtained from P_2O_5 and 99.8% D_2O. This showed only one aromatic H peak at 7.33 ppm. The ratio of its integrated band area to that of the phenolic hydroxyl H showed it to be equivalent to one H.

When I was deuterated at 95°, the product isolated and purified from aliquots obtained at different time intervals showed the almost complete disappearance of the AB quartet (Figure 2b) indicating that H's at 8 and 9 were the first to be exchanged 15 min after reaching 95°. Two hours later, about 40% of the H at position 1 had exchanged with ^{2}H (Figure 2d) as shown by comparison of the integrated areas of the hydroxyl protons with that of H-1. At 3 hr, less than 5% still remained unexchanged while a sharp peak appeared at 7.33 ppm and a relatively broad peak at 7.54 ppm (Figure 2e). Finally (aliquot 6), after raising the temperature to

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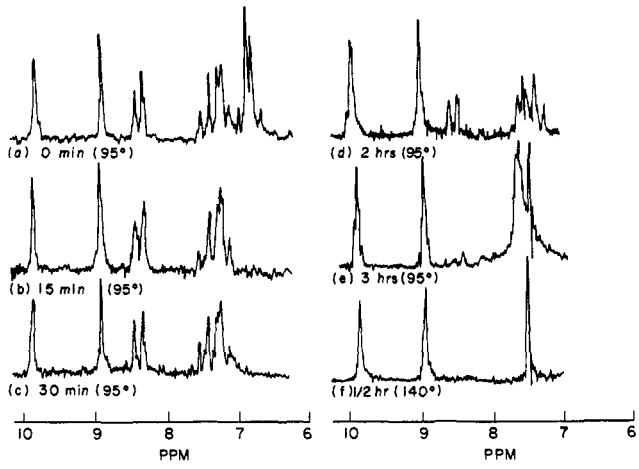
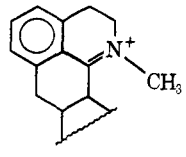


Figure 2. The 60-MHz pmr spectra of apomorphine deuterated at 95 and 140° for various time intervals.

140° and maintaining it for 0.5 hr, one band, equivalent to one hydrogen, was present at 7.33 ppm (Figure 2f).

The mass spectrum of the hydrochloride of I showed a strong peak at *m/e* 267 (molecular ion M for I) indicating that during mass spectroscopy HCl was removed. A total of seven peaks were observed in association with M (Figure 3a). An intense M − 1 peak probably corresponds to the following ion.[17]



By contrast, the mass spectrum of deuterated I (from aliquot 6) showed a total of 11 lines associated with *m/e* 267 with the highest intensity at *m/e* 271 (Figure 3b). Assuming that the probability of hydrogen and deuterium loss is equal (*i.e.*, no isotopic effects), the per cent composition of labeled and unlabeled I, after correcting for the contributions of ^{13}C and M − *n* peaks from the mass spectrum of I, was found to be: d_0, 23; d_1, 0; d_2, 11; d_3, 19; d_4, 41; d_5, 6.0.

The pmr spectrum of this sample (Figure 2f) corresponds essentially to I-d_4. The presence of I-d_3, I-d_2, and I-d_0 shows that the exchange between deuterium on the aromatic rings and HCl had taken place during vaporization in the spectrophotometer. This mass spectrum, therefore, can be relied upon to indicate only the maximum amount of deuterium in the molecule.

Discussion

The preparation of VI directly from I offered the following advantages: (a) yields tended to be higher; (b) fewer impurities arose during the reaction; (c) greater commercial availability of the starting material I; (d) reaction could be kinetically controlled to yield VI labeled on ring D or both aromatic rings.

The last point is significant, for it affords an indirect method for demonstrating whether I is enzymatically hydroxylated on either one or both rings provided that the tritium label does not exchange in a biological system as our results reported elsewhere have shown.[18] In addition, others have shown that 3,4-dihydroxyphenylalanine-*2,5,6*-t_3 remains stable in the mouse after 2 days.[19]

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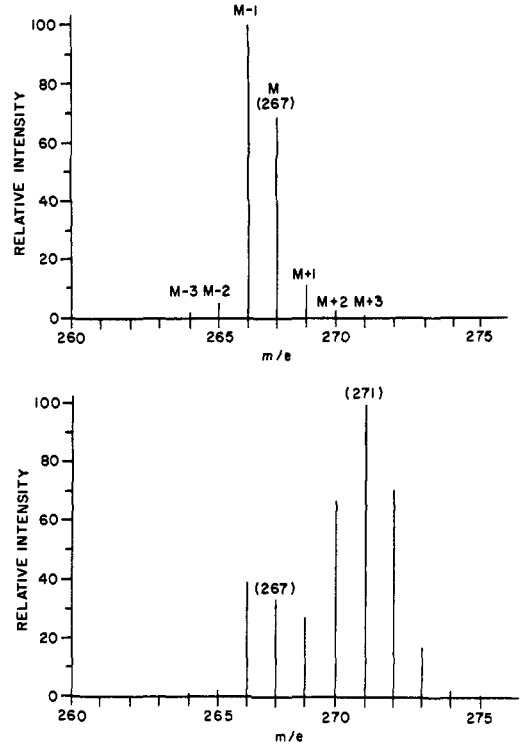

Figure 3. (a, top) Mass spectrum of the molecular ion fragment of apomorphine; (b, bottom) mass spectrum of the molecular ion fragments of deuterated apomorphine.

The combined pmr and mass spectrometric results of deuterated I show that in the tritiation of I only aromatic hydrogens are exchanged to any large extent, and although the statistical distribution of tritium at equilibrium is not known, the large difference in exchange rates between the two aromatic rings indicates that by modifying the reaction conditions one may obtain VI labeled predominantly, if not exclusively, on the catechol ring.

In accordance with theory, the pmr studies of partially deuterated I showed that the most easily exchangeable aromatic H's were those at positions 8 and 9 of ring D. In an electrophilic reaction, such as the present exchange reaction,[20] the existence of electron-releasing functions at 10 and 11 enhances the electron density of 8 and 9 thus favoring attacks by electrophilic reagents at those positions.[21] On the other hand, the diminished relative exchange rates for positions 1, 2, and 3 as indicated by pmr (Figure 2) paralleled the relative electron densities for these positions as estimated by an extended Hückel-type LCAO–MO method[22] and other mathematical treatments on biphenyl.[23] Position 2, for which an electron deficit

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relative to 1 and 3 has been calculated,[22] was shown to be the least reactive to electrophilic exchange. In contrast to H's 1 and 3, the band at 7.33 ppm, which is closest to the frequencies assigned to H-2 remained unchanged in intensity even after 1 hr at 140°.

Experimental Section

Pmr spectra were obtained at room temperature in DMSO-d_6 using tetramethylsilane as an internal standard on a Varian 60-A nmr spectrometer, with chemical shifts expressed in ppm and coupling constants, J, in Hz. A Perkin-Elmer R12B spectrometer was used for double irradiation experiments.

Mass spectra of I and [^{2}H]apomorphine were obtained with a Hitachi RMU-7 mass spectrometer; ion potential, 80 V; inlet temperature *ca.* 200° (direct solid inlet).

Tritium radioactivity measurements were carried out in Aquasol with [^{3}H]toluene as an internal standard in a Beckman LS-233 liquid scintillation counter. Specific activities were computed as the averages of four determinations of 1-ml aqueous solutions of [^{3}H]apomorphine hydrochloride in 10 ml of Aquasol.

Spectrophotofluorimetric[18,24] determinations of emission spectra and concentrations were carried out in aqueous solutions (triple-distilled water) using a maximum excitation wavelength, 376 nm, and maximum emission wavelength, 450 nm. The concentrations ranged up to 1 μg/ml where Beer–Lambert's law was found to hold; intensities were corrected for pH effects. An Aminco Bowmann spectrofluorimeter, Model 34008-27, was used equipped with a xenon compact arc lamp and a X–Y recorder.

Thin layer chromatography was performed with (A) 1-butanol–water–acetic acid (4:1:1) and (B) 1-butanol–water–acetic acid–pyridine (30:24:6:20) on Eastman Organic silica gel 6060 Chromagram sheets with apomorphine hydrochloride as the standard. Exposure to I_2 vapors oxidized the compound to a characteristic green color.

Radiochemical purity was checked autoradiographically by exposing Eastman-Kodak Med. X-ray film, NS54T, to the chromatograms up to a month.

Materials were tritiated water (sp activity 1 Ci/ml), International Chemical and Nuclear Corp.; deuterated water (99.8%), AEC, Savannah River; phosphorus pentoxide, analytical reagent (99.0% as P_2O_5), Mallinckrodt; phosphoric acid, Fisher certified reagent (85% as H_3PO_4); apomorphine hydrochloride (purity $\geq$98.5%), Merck; morphine sulfate, Eli Lilly & Co.; sodium sulfate, anhydrous, reagent ACS (98.5% as Na_2SO_4), J. T. Baker Chemical Co.; Aquasol, New England Nuclear; [^{3}H]toluene (sp act 2.06×10^6 dpm/ml), New England Nuclear.

A. **[^{3}H]Apomorphine (VI) from Morphine** (specific activity $3.62 \pm 0.03 \times 10^3$ dpm/μg). The free base of morphine (1.94 g, 6.8 mM) obtained by treating an aqueous solution of morphine sulfate with $NaHCO_3$ was added to 11.2 ml of 85% phosphoric acid containing 0.10 ml of tritiated water at 125–130° with stirring and heating in an oil bath while nitrogen was bubbled through at a brisk rate. The pot temperature was raised to 140° over a period of 20 min and maintained for 1 hr. During this time the suspended morphine went into solution gradually, the color changing to dark brown. No reflux condenser was used in order to allow continuous removal of water vapor. At the end of the reaction, the temperature was allowed to drop to 80°, with continuous passage of nitrogen, followed by the transfer of the contents to a glass container with the addition of 38 ml of water. After the solution was cooled overnight to 4° with no visible precipitation, a crude, tan-colored solid was precipitated by the addition of excess NaCl. The precipitate was centrifuged and redissolved in *ca.* 55 ml of water at 55–60°. The solution, cooled to ice–water temperature, was treated with excess Na_2SO_3, and the resultant precipitate was centrifuged and dried under high vacuum and over P_2O_5. The crude free base of VI was removed with *ca.* 300 ml of ether by continuous extraction overnight. After its volume was reduced to *ca.* 60 ml under reduced pressure, the ether extract was treated with *ca.* 50 ml of absolute ether saturated with gaseous HCl. The white flocculent salt of VI was centrifuged off and dried under high vacuum and over P_2O_5 to a constant weight, 0.362 g (17% yield). The product was twice dissolved in *ca.* 15 ml of methanol and precipitated with excess absolute ether yielding a product with a specific activity of $3.79 \pm 0.05 \times 10^3$ dpm/μg. Finally, the compound was lyophilized twice from water, losing successively 0.012 and 0.003% of its radioactivity. The specific activity of the final product was $3.62 \pm 0.03 \times 10^3$ dpm/μg. The emission spectrum was identical with that of I (Merck), while purity based on spectrofluorimetric measurements was found to be 98% ± 5%: tlc R_f (A) 0.59, R_f (B) 0.72 (identical with standard); autoradiography (exposure, 1 month) single spots, R_f (A) 0.59, R_f (B) 0.72.

B. **VI from Morphine** (specific activity $1.65 \pm 0.01 \times 10^5$ dpm/μg). Tritiated 85% phosphoric acid was prepared by adding, slowly, 14.5 ml of tritiated water (1 Ci/ml) to 23.3 g of P_2O_5 in a 25-ml round-bottom flask placed in an ice–water bath and equipped with a reflux condensor vented through a drying tube filled with Drierite, and a dropping funnel with a pressure-equalizing side arm. When most of the P_2O_5 had dissolved, the ice bath was removed and agitation was continued with a magnetic stirrer. The rearrangement of the free base of morphine (3.89 g, 0.0137 mol) to VI was carried out as described in part A with the additional precautionary measure of trapping vapors in two Dry Ice–acetone traps connected in series because of the high radioactivity involved (14 Ci). The product was lyophilized repeatedly until loss of radioactivity was reduced to 0.02%, 192 mg (9% yield), specific activity $1.67 \pm 0.1 \times 10^5$ dpm/μg. Tlc, autoradiography (exposure, 1 and 3 weeks) and spectrophotofluorimetry yielded results identical with those of part I.

C. **VI from I** (specific activity $1.88 \pm 0.02 \times 10^5$ dpm/μg). Tritiated 85% phosphoric acid was prepared as described in part B. P_2O_5 (16 g) was converted to 85% H_3PO_3 by the addition of 10.0 ml of tritiated water (1 Ci/ml). After bubbling nitrogen through the solution for 10 min, the temperature was raised to 132° with the aid of a heating mantle followed by the addition of I (1.72 g, 5.7 mmol). The temperature was raised and maintained at 141 ± 2° for 1 hr during which time the solution color remained light amber. The reaction was worked up in the manner described above. However, instead of using continuous ether extraction for the isolation of the free base, it was found that extraction from an aqueous phase was more effective. The crude product was extracted with 4 × 50 ml of ether from 70 ml of aqueous solution. The combined ether extracts were dried over anhydrous Na_2SO_4, filtered, and reduced to *ca.* 40 ml to which 40 ml of gaseous HCl–saturated absolute ether was added. The white flocculent precipitate was dried under high vacuum over P_2O_5 to a constant weight, 353 mg (21% yield). After loss of activity was reduced to 0.002%, by repeated lyophilization, the specific activity was determined to be $1.88 \pm 0.02 \times 10^5$ dpm/μg. Tlc, autoradiography (exposure 1 week), and spectrophotofluorimetry yielded the results identical with those of part I.

D. **[^{2}H]Apomorphine Hydrochloride from Morphine.** [^{2}H]-Apomorphine hydrochloride was prepared in the same manner as VI described in part B. The base of morphine sulfate (4.0 g) was allowed to act in 85% D_3PO_4 prepared from 23.3 g of P_2O_5 and 14.5 ml of D_2O (99.8%). The weight of final product was 450 mg (11% yield). Tlc yielded single spots with R_f values identical with those of I. Pmr (after lyophilization): NCH_3 3.07; aromatic H (one sharp absorption band) 7.33; phenolic hydroxyl H's (slightly broad) 8.87, 9.83 (disappear on addition of D_2O); acidic H 4.30 (broad peak); aliphatic H's between 4.87 and 2.73 (complex pattern); ratio of H's 3:1:1:1.

E. **[^{2}H]Apomorphine Hydrochloride Prepared from I.** I (5.0 g) was dissolved in D_3PO_4 at 70–74° prepared from 47.0 g of P_2O_5 and 29 ml of D_2O (99.8%) with N_2 bubbling through the solution during the reaction. The temperature was raised and maintained at 95°. Five aliquots (*ca.* 8 ml each) were removed at time intervals of 10, 15, and 45 min, 2 and 3 hr from the moment temperature reached 95°. Finally, the remainder (sixth aliquot) was heated to 140° for 0.5 hr before it was removed. All aliquots were worked up in the manner described in parts A and B. Pmr spectra were obtained for aliquots 1, 2, 4, 5, and 6: aliquot 6, pmr spectrum was identical with that of part D; aliquot 1, spectrum identical with that of standard I minus AB quartet assigned to H's 8 and 9; aliquot 2, spectrum was essentially identical with that of aliquot 1; aliquot 5, spectrum shows trace absorption of H at 1, at 8.43 a sharp band at 7.33, and a band at 7.60 ppm with a broadened base.

Acknowledgment. We are grateful to Dr. David Christman for obtaining the mass spectra and Dr. Lewis Friedman for his valuable interpretation and guidance in the use of the mass spectra.

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